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Characterization of SPECTRUM Variable Nicotine Research Cigarettes

Patricia Richter, PhD [Deputy Branch Chief],
Tobacco and Volatiles Branch, Centers for Disease Control and Prevention, Atlanta, GA

Pappas R. Steven, PhD [Team Lead],
Tobacco and Volatiles Branch, Centers for Disease Control and Prevention, Atlanta, GA

Roberto Bravo, PhD [Team Lead],
Tobacco and Volatiles Branch, Centers for Disease Control and Prevention, Atlanta, GA

Joseph G. Lisko, MS [Team Lead],
Tobacco and Volatiles Branch, Centers for Disease Control and Prevention, Atlanta, GA

Maria Damian, MS [Chemist],
Battelle Analytical Services, Atlanta, GA

Nathalie Gonzalez-Jimenez, BS [Chemist],
Oak Ridge Institute for Science and Education, Oak Ridge, TN

Naudia Gray, MS [Chemist],
Tobacco and Volatiles Branch, Centers for Disease Control and Prevention, Atlanta, GA

Lisa M. Keong, BS [Chemist],
Battelle Analytical Services, Atlanta, GA

Jacob B. Kimbrell, BS [Chemist],
Oak Ridge Institute for Science and Education, Oak Ridge, TN

Peter Kuklenyik, PhD [Tobacco and Volatiles Branch],
Centers for Disease Control and Prevention, Atlanta, GA

Tameka S. Lawler, MPH [Chemist],
Tobacco and Volatiles Branch, Centers for Disease Control and Prevention, Atlanta, GA

Grace E. Lee, MPH [Chemist],
Tobacco and Volatiles Branch, Centers for Disease Control and Prevention, Atlanta, GA

Magaly Mendez, MPH [Chemist],
Tobacco and Volatiles Branch, Centers for Disease Control and Prevention, Atlanta, GA

Jose Perez, BS [Chemist],

Correspondence Dr Pappas; RPappas@cdc.gov.

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Tobacco and Volatiles Branch, Centers for Disease Control and Prevention, Atlanta, GA

Shakia Smith, BS [Chemist],
Oak Ridge Institute for Science and Education, Oak Ridge, TN

Hang Tran, MS [Chemist],
Tobacco and Volatiles Branch, Centers for Disease Control and Prevention, Atlanta, GA

Robert Tyx, PhD [Biologist], and
Tobacco and Volatiles Branch, Centers for Disease Control and Prevention, Atlanta, GA

Clifford H. Watson, PhD [Lab Chief]
Tobacco and Volatiles Branch, Centers for Disease Control and Prevention, Atlanta, GA

Abstract

Objective—To provide researchers an extensive characterization of the SPECTRUM variable nicotine research cigarettes.

Methods—Data on cigarette physical properties, nicotine content, harmful and potentially harmful constituents in the tobacco filler was compiled.

Results—Data on physical properties, concentrations of menthol, nicotine and minor alkaloids, tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons, ammonia, and toxic metals in the filler tobacco for all available varieties of Spectrum research cigarettes are provided. The similarity in the chemistry and physical properties of SPECTRUM cigarettes to commercial cigarettes renders them acceptable for use in behavioral studies. Baseline information on harmful and potentially harmful constituents in research tobacco products, particularly constituent levels such as minor alkaloids that fall outside typical ranges reported for commercial, provide researchers with the opportunity to monitor smoking behavior and to identify biomarkers that will inform efforts to understand the role of nicotine in creating and sustaining addiction.

Conclusions—Well characterized research cigarettes suitable for human consumption are an important tool in clinical studies for investigating the physiological impacts of cigarettes delivering various levels of nicotine, the impact of reduced nicotine cigarettes on nicotine addiction, and the relationship between nicotine dose and smoking behavior.

Keywords

tobacco; cigarettes; spectrum; nicotine; alkaloids; metals; PAH

INTRODUCTION

The 22nd Century Group, Inc. uses a combination of techniques including genetic engineering and plant breeding to provide cigarette tobacco filler with reduced nicotine content. The 22nd Century Group manufactured variable nicotine cigarettes under subcontract with the Research Triangle Institute (RTI).[1] The modified nicotine cigarettes include a minimal (placebo) nicotine delivery level, menthol and non-mentholated varieties, and eight target nicotine delivery levels. The products also differ in tar deliveries, ventilation, and levels of other constituents. A total of 23 cigarette configurations are made

available to researchers through NIDA's Drug Supply Program under the name, "SPECTRUM."[1,2]

Unlike other research tobacco products, SPECTRUM cigarettes provide researchers with a variable nicotine cigarette that is "suitable" for smoking by human test subjects.[3] Some research incorporating SPECTRUM cigarettes has already been published and more is expected due to multiple funding opportunities intended to facilitate research that will help inform the U.S. Food and Drug Administration in priority research areas including dependence thresholds and the impact of nicotine reduction on tobacco product use behavior.[3–5]

Nicotine addiction and its relation to smoking behavior is complex. The manner in which a smoker utilizes the product and levels of chemical constituents in tobacco and smoke determine the exposure to smoke constituents.[6] In addition to general considerations of toxicity, some tobacco smoke constituents like acetaldehyde[7] are also thought to be a factor in addiction. Therefore additional data on the characterization of the SPECTRUM cigarettes will inform researchers using variable nicotine cigarettes in behavioral, addiction, exposure biomonitoring, cessation, and other tobacco-related studies.

We report here an extensive characterization of physical properties and constituent levels for SPECTRUM variable nicotine research cigarettes. This characterization includes the physical properties of the cigarettes (length, pressure drop, filter ventilation, filter circumference, rod circumference, filter length, tobacco length, over wrap length, air permeability, and tobacco weight and cigarette weight) as well as menthol, nicotine, minor alkaloids, and several major classes of harmful and potentially harmful organic and inorganic chemical constituents in the tobacco.

METHODS

Twenty-three varieties of SPECTRUM research cigarettes were obtained from NIDA (Bethesda, MD, USA) in 2014 and stored in original packaging after receipt.

Results are reported on an "as received" basis unless otherwise noted.

Physical properties

After conditioning the cigarettes according to ISO 3402 (1999) specifications, physical property determinations were performed using a C2 instrument (Cerulean, Milton Keynes, UK). Five replicate measurements were made on each cigarette variety to determine cigarette length, pressure drop, filter ventilation, filter circumference, rod circumference, filter length, tobacco length, and over wrap length. Air permeability was determined using a PPM1000M instrument (Cerulean, Milton Keynes, UK).

Tobacco filler and cigarette weights were determined manually with calibrated and certified balances.

Quantitative analytical measurements

All quantitative methods were performed in a strict QA/QC environment. Each analytical method below has been full validated and has sufficient dynamic range for all samples. The limits of detections for each method was obtained using Taylor's method.[8] All reported data fall between the lowest and highest calibration curve point. Any measured values lower than the lowest calibration point are reported as non-detects. All run QC were checked using a modified Westgard protocol.[9] Any QC failures were excluded and samples repeated if needed. All data were reviewed by a quality control officer and quality manager prior to release.

Menthol

Previously unopened packages of cigarettes were used for menthol analysis to minimize substantial evaporative losses. Sample preparation procedures were described previously.[10] Tobacco menthol levels were determine analytically using an Agilent 7890 GC coupled with a 5975 MSD (Agilent Technologies; Newark, DE, USA). The chromatographic separation was achieved using an Ultra-2 capillary column (25m × 0.32mm × 0.52μM, Agilent Technologies; Andover, MA, USA) with research grade helium carrier gas as previously described.[10] Results are the mean and standard deviations of five replicate analyses.

Tobacco specific nitrosamines

Tobacco filler from individual cigarettes was removed from the paper wrapper and ground to uniformity with a Cuisinart® Spice and Nut grinder. For each cigarette type, five 0.25 g aliquots of the ground filler were measured into 15 mL amber vials and spiked with 100 μL internal standard solution, representing 100 ng ^{13}C labeled standard for each analyte. Ten mL of 100 mM aqueous ammonium acetate solution was added to each sample and shaken for 60 minutes at 250 rpm on a Benchmark IncuShaker 10 L (Benchmark Scientific, Edison, NJ, USA) Approximately 0.5 mL of the extract was pipetted into a Thompson Filter Vial and press-filtered at 0.45 μm.

Analytical separation was performed on an Agilent 1200 G1312B binary pump. (Agilent Technologies, Santa Clara, CA) using a 5μm particle size Xterra MS C18 4.6 × 50mm HPLC column (Waters Corporation, Milford, MA, USA) with mobile phase pumped at 1 mL/min at 60°C. Elution began with 95% solution A (5mM aqueous ammonium acetate), 5% solution B (95% acetonitrile, 5% water with 5mM ammonium acetate) mobile phase for 1 min, followed by a 1 min gradient to 65% solution A, 35% solution B. The mobile phase was maintained at 65% solution A, 35% solution B for 3 min, followed by a 2 min gradient to the original mobile phase. Analytes were quantitated with an API 4000 triple quadrupole mass spectrometer (Sciex Corporation, Farmington MA, USA) working in positive electrospray ionization mode as previously described.[11] Tandem mass spectrometry data was evaluated and quantitated using Sciex Analyst 1.5 software.

Alkaloids

Analyses of alkaloids were performed using an Agilent 7890 GC coupled with a 7000 Triple-Quad detector (Agilent Technologies, Newark, DE, USA). The chromatographic separation was accomplished using a DB-1701 capillary column (30m × 0.250 μM, 0.25 μM,

J&W Scientific) with research grade helium used as the carrier gas as previously described.[12] Results are the mean and standard deviations of five replicate analyses (N = 5).

Polycyclic Aromatic Hydrocarbons

Tobacco filler from individual cigarettes were weighed, spiked with an isotopically labeled13C-PAH mixture as an internal standard, extracted with 10 ml cyclohexane followed by evaporation to 1 ml. Extracts were filtered through a 0.45 μM Nylon syringe filter. The PAHs were subsequently analyzed by SIM GC/MS, normalized to tobacco weight and expressed in nanograms of analyte per gram tobacco. Analytes measured included acenaphthene (ACE), acenaphthylene (ACL), anthracene (ANT), benzo[a]pyrene (BAP), benzo[e]pyrene (BEP), chrysene (CHR), fluoranthene (FLR), fluorene (FLU), naphthalene (NAP), phenanthrene (PHE), pyrene (PYR), benz[a]anthracene (BAA), benzo[b]fluoranthene (BBF), and benzo[k]fluoranthene (BKF). Lowest reportable levels were determined as the concentration of the lowest standards for each analyte. Five replicates were analyzed for each variety.

Nicotine

Tobacco filler from individual cigarettes was removed from the paper wrapper, 5 mL of 2N NaOH was added to 1.0 grams of tobacco filler in a 60 mL amber vial, followed by extraction with 50 mL of an MTBE stock that contained quinoline as an internal standard. A 1 mL aliquot of the extract was placed in a 2 mL amber vial for GC/MS analysis. The sample preparation and nicotine analytical method have been previously described.[13] Five replicates were analyzed for each variety.

Ammonia

Tobacco filler from individual cigarettes was removed from the paper wrapper, placed into a 60-mL amber bottle, and weighed. Deionized water (30 mL) was added and the sample was sealed with a PTFE-lined cap. The sample was shaken for 30 min at 160 rpm on a Max Q 2000 shaker (Barnstead/Labline, Dubuque, IA, USA). A 1.5mL sample aliquot was centrifuged in a Sorval Pico Biofuge (Thermo Fisher, Waltham, MA, USA) at 13,000 rpm for 5 min to remove tobacco debris. A 10:1 water to sample dilution was made in a 1.5 mL sample vial. The sample was vortexed and placed in a sample tray for analysis. Five replicates were analyzed for each variety.

An ISO Guide 34 1000 mg/L ammonium standard was purchased from Sigma Aldrich (St. Louis, MO, USA). Ultrapure water used in all procedures was from an Aqua Solutions water system (Aqua Solutions, Jasper, GA, USA).

Samples (25 μL injections) were analyzed with a Dionex ICS-3000 chromatography system with Chromeleon version 6.8 software and a 4 mm Ion Pac CS12A cation exchange column (Thermo Scientific, Sunnyvale, CA, USA). Isocratic separation of the ammonium ion was achieved using a 20 mM methanesulfonic acid eluent prepared by the EG40 eluent generator with cation self-regenerating suppressor that required only an ultrapure water source in order to suppress the conductance from the eluent (Thermo Scientific, Sunnyvale, CA USA). The

column temperature was maintained at 25°C with a run time of 15 minutes. Ammonium ions were detected using a conductivity detector.

Inorganic Constituents

Tobacco filler from 4 individual cigarettes of each variety was removed from the paper wrapper and combined for analysis. The tobacco was dried for 3 hours at 90°C and rendered more homogeneous by grinding for 20 seconds with a Smart Grind coffee grinder (Black and Decker, Middleton, WI, USA). Samples (0.100 to 0.150 g) were digested with a Milestone Ethos microwave system and analyzed with “Triple Quad” Inductively Coupled Plasma Mass Spectrometry as previously described.[14] Dried tobacco was analyzed for mercury using a combustion mercury analyzer as previously described[15] with minor modification.[16] Quality control was maintained throughout using Reference tobacco 1S3 (North Carolina State University, Raleigh, NC, USA) and certified reference material (CRM) CTA-OTL-1 (Instytucie Chemii i Techniki Jadrowej, Warszawa, Poland) as previously described.[14,16] Results are reported as the mean and standard deviations of 5 replicate analyses.

RESULTS

Physical Properties

Based on physical properties, the cigarette paper seemed consistent for all SPECTRUM varieties. Cigarette diameter, circumference, tip length (Table 1), and paper porosity differed negligibly from variety to variety. The average paper porosity for all SPECTRUM cigarette varieties was 31.5 ± 3.7 CORESTA units. All measured physical properties are in Tables 1 and 2.

Menthol Results

Menthol concentrations varied greatly among the SPECTRUM cigarettes. Twelve mentholated products (NRC101, NRC103, NRC105, NRC201, NRC301, NRC302, NRC401, NRC405, NRC501, NRC601, NRC602, and NRC701) had menthol concentrations ranging from 4970 – 7540 μg menthol/cigarette. Non-mentholated products had measured menthol concentrations ranging from 1.1 – 114.0 μg/cigarette. Both of these ranges were consistent with menthol concentrations recently reported for commercial menthol and non-menthol cigarette brands sold in the United States.[17] Results are reported in Table 3.

Nitrosamines

Results for NNN (N-nitrosonornicotine) and NNK (4-(N-nitrosomethylamino)-1-(3-pyridyl)-1-butanone) were reported in Table 3. NNN concentrations ranged from 963 ng/g to 1732 ng/g, NNK values were 45.2 ng/g to 517 ng/g. As expected, concentrations of NNN in the tobacco filler were higher than that of NNK. These results place the NNN levels of SPECTRUM cigarettes within the lower third of the concentration range reported for commercial cigarette products.[18] The majority of SPECTRUM NNK values were lower than the lowest NNK level (330 ng/g) published by Stepanov et al.[18]

Polycyclic Aromatic Hydrocarbons

Although PAHs are primarily combustion products, difference in tobacco processing, ie, curing practices, can result in PAHs being present in tobacco filler. The results for analyses of PAHs in the tobacco from SPECTRUM cigarettes are reported in Table 4. Of the 14 PAHs analyzed, 8 PAH compounds were present at detectable levels ranging from 0.57 – 349 ng/g. No detectable levels of BAP, ACL, FLU, ANT, BAA, or NAP were found in the SPECTRUM cigarettes. The highest PAH concentrations were found for CHR (193 – 349 ng/g) and PHE (32.7 – 59.7ng/g), which were detected in all of the products analyzed. The compounds PYR (8.47 – 16.6 ng/g) and FLR (7.78 – 11.8 ng/g) had lower levels but were detectable in all of the varieties tested. Little data on PAH concentrations in cigarette filler tobacco has been published since fire-cured tobacco is not commonly used for filler. However, phenanthrene and chrysene, concentrations in all Spectrum variety tobacco fillers were higher than the respective concentrations determined in 3R4F (used as a quality control, 26.3 ng/g and 3.7 ng/g, respectively), whereas fluoranthene, benzo[e]pyrene and benzo[a]pyrene concentrations were lower in all Spectrum variety tobacco fillers than in 3R4F (15.7 ng/g, 2.0 ng/g, and 1.3 ng/g, respectively).

Nicotine and Minor Alkaloids

The results of analyses for nicotine and minor alkaloids (nornicotine, myosmine, anabasine, anatabine, and isonicotine are reported in Table 5. Nicotine concentrations ranged from very low to within the range reported for commercial products (0.28 – 25.0 mg/g). The majority of products had nicotine concentrations that were well below the levels of comparable commercial cigarette products,[19] which typically range from 8 – 22 mg/g, indicating a selective reduction of nicotine. Overall concentrations of other alkaloids were higher than those found in commercial products[12] and generally increased with measured nicotine concentration for each SPECTRUM product type. Alkaloid concentration ranges for the SPECTRUM varieties were 472 – 1235 μg/g for nornicotine, 14.8 – 28.7 μg/g for myosmine, 5.9 – 242.4 μg/g for anabasine, 29.7 – 2124 μg/g for anatabine and 3.2 – 64.9 μg/g for isonicotine.

Inorganic Analytes

Ammonia levels in the tobacco filler differed little between the cigarette varieties, ranging from 2.2 to 3.7 mg ammonia per gram of conditioned tobacco filler (Table 6). Within-variety replicate measurements showed little variation with relative standard deviations ranging from 1.6 to 5.6 %. This range is in line with previously reported ammonia concentrations in tobacco filler.[20]

The results for 11 inorganic harmful and potentially harmful constituents of SPECTRUM cigarette tobacco filler showed that concentration ranges for beryllium, nickel, and arsenic in filler tobacco from SPECTRUM cigarettes (Table 6) were lower than the respective ranges of concentrations in filler tobacco previously reported for 50 varieties of cigarettes commercially available in the United States.[15] Chromium, cobalt, and cadmium concentrations in SPECTRUM filler tobacco (Table 6) were below, or corresponded with the lower range of chromium, cobalt, and cadmium concentrations previously reported.[15] Manganese and lead concentrations in SPECTRUM cigarette filler tobacco fell

predominantly within the ranges of manganese and lead concentrations previously reported.[15]

DISCUSSION

Reference materials for analytical or clinical purposes are extremely useful to provide a consistent measurement process and to test various hypotheses. For example, when a measurement result for a reliable reference material is within the range specified for a specific property of the material, valid inferences may be made regarding the accuracy of measurements of that property when using the same method for measurements of uncharacterized samples with the same matrix. Reference materials may thus be utilized to infer that laboratories that can produce acceptable measurement results with a specific reference material are intercalibrated with other laboratories that have also been able to produce acceptable results.[21,22] Since 1968, 17 Monitor quality control cigarettes, "reference" cigarettes and smokeless tobacco products have been produced and made available to researchers by CORESTA and the University of Kentucky for analytical chemistry and toxicology testing.[23–25] In addition, 9 smokeless tobacco "reference" products are currently distributed by North Carolina State University.[26] Manufacturers and related groups provide limited characterization of the design and chemistry of the quality control and reference products.[24–29] These reference and monitor products are an invaluable resource to the research community engaged in analytical chemistry method development, chemical analysis of tobacco and tobacco smoke, and toxicological tobacco product research. However, tobacco reference products are produced from tobacco, an agricultural product, and, consequently, they are unlikely to be sufficiently characterized, stable, and homogenous in nature to meet the definition of a Standard Reference Material.[21] However these products have a long history of use as quality control materials and have been analyzed and reported in the scientific literature alongside commercial products.[27–29]

Reference tobacco products are typically accompanied by a disclaimer that they are not for human consumption.[25,26] Because of this restriction, researchers use commercial tobacco products in clinical studies requiring subjects to smoke tobacco cigarettes or use smokeless tobacco. Manufacturers can remove commercial tobacco products from the market place without notice and are not required to publicly disclose the chemical constituents in their products. Also, only a limited number of commercial products have been available to study cigarettes that differ substantially in nicotine content.[30]

Well characterized research cigarettes suitable for human consumption are an important tool in clinical studies such as those investigating the physiological impacts of cigarettes delivering various levels of nicotine, the combined impact of nicotine replacement and reduced nicotine cigarettes on nicotine addiction, and the relationship between nicotine dose and smoking behavior. In the work reported here we observe that most constituents, including the flavor compound menthol, are at or near the levels typically reported for commercial U.S. cigarette brands. Nicotine measurements confirm the targeted reduction of nicotine in the tobacco filler. Concentrations of other tobacco alkaloids were not likewise reduced. For example, while NNN and NNK were at the lower end of values reported for commercial products,[18] overall concentrations of minor tobacco alkaloids that are precursors

for formation of TSNAs in a burning cigarette,[12,28] were higher than those found in commercial products. It has also been suggested that the minor tobacco alkaloids interact with the reinforcing properties of nicotine and thus may influence smoking behavior.[32]

The overall similarity in the chemistry and physical properties of SPECTRUM cigarettes to commercial cigarettes has potential implications for the acceptability and use behavior of study participants using the products. Baseline information on harmful and potentially harmful constituents in reference tobacco products, particularly constituent levels that fall outside typical ranges reported for commercial products, provide researchers with the opportunity to monitor smoking behavior and to identify biomarkers that will inform efforts to understand the role of nicotine in creating and sustaining addiction.

IMPLICATIONS FOR TOBACCO REGULATION

The large amount of analytical data on Harmful and Potentially Harmful Constituents (HPHCs) in the filler tobacco in SPECTRUM cigarettes presented here will supplement data on concentrations of already reported directly to the FDA center for Tobacco Products and in published manuscripts.[14–17,28] In addition, the concentrations of nicotine and related alkaloids reported here will better enable researchers to interpret data in smoking topography and addiction studies. These data together with topography and addiction studies will better inform regulatory agencies when making decisions on regulatory levels for HPHCs and on the impact of reduced nicotine levels on potential for product addictiveness and cessation success.

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Table 1

SPECTRUM Cigarette Physical Parameters

Product Code	Cigarette Mass (mg)	Diameter (mm)	Circumference (mm)	Filter Length (mm)	Tip Length (mm)	Overwrap Length (mm)
NRC100	970 ± 18	7.93 ± 0.03	24.93 ± 0.08	22.6 ± 0.3	30.3 ± 0.3	7.7 ± 0.1
NRC101	934 ± 31	7.96 ± 0.01	25.00 ± 0.04	22.6 ± 0.6	30.3 ± 0.5	7.7 ± 0.1
NRC102	897 ± 20	7.95 ± 0.03	24.98 ± 0.09	22.7 ± 0.6	30.4 ± 0.7	7.7 ± 0.1
NRC103	913 ± 25	7.92 ± 0.02	24.88 ± 0.06	22.8 ± 0.4	31.2 ± 0.9	8.4 ± 1.3
NRC104	946 ± 28	7.91 ± 0.01	24.83 ± 0.03	25.1 ± 1.8	30.5 ± 0.1	5.4 ± 1.8
NRC105	898 ± 22	7.91 ± 0.01	24.86 ± 0.03	23.0 ± 0.5	30.3 ± 0.1	7.4 ± 0.5
NRC200	939 ± 35	7.93 ± 0.01	24.93 ± 0.02	22.6 ± 0.2	30.1 ± 0.1	7.5 ± 0.1
NRC201	933 ± 59	7.94 ± 0.02	24.95 ± 0.08	22.8 ± 0.4	30.4 ± 0.3	7.7 ± 0.1
NRC300	979 ± 21	7.94 ± 0.01	24.96 ± 0.04	22.7 ± 0.2	30.8 ± 1.5	8.0 ± 1.6
NRC301	890 ± 16	7.96 ± 0.01	25.00 ± 0.02	22.3 ± 0.6	30.7 ± 1.2	8.4 ± 1.2
NRC302	894 ± 9	7.87 ± 0.02	24.73 ± 0.07	24.9 ± 1.7	30.4 ± 0.3	5.5 ± 1.7
NRC400	969 ± 20	7.95 ± 0.01	24.97 ± 0.05	22.6 ± 0.3	31.9 ± 2.0	9.3 ± 2.2
NRC401	944 ± 21	7.89 ± 0.02	24.77 ± 0.06	22.5 ± 0.3	30.9 ± 1.6	8.5 ± 1.3
NRC402	950 ± 32	7.90 ± 0.01	24.83 ± 0.04	23.0 ± 0.7	30.3 ± 0.1	7.3 ± 0.7
NRC404	915 ± 33	7.93 ± 0.01	24.91 ± 0.04	23.2 ± 0.1	30.5 ± 0.7	7.3 ± 0.8
NRC405	875 ± 48	7.89 ± 0.01	24.79 ± 0.03	23.0 ± 0.4	30.4 ± 0.1	7.4 ± 0.5
NRC500	935 ± 29	7.90 ± 0.01	24.81 ± 0.02	22.5 ± 0.5	30.1 ± 0.2	7.5 ± 0.6
NRC501	975 ± 22	7.93 ± 0.02	24.91 ± 0.05	22.5 ± 0.2	30.2 ± 0.4	7.7 ± 0.4
NRC600	973 ± 35	7.90 ± 0.02	24.81 ± 0.06	25.1 ± 2.1	31.3 ± 1.6	6.2 ± 2.1
NRC601	936 ± 16	7.91 ± 0.02	24.86 ± 0.05	23.3 ± 0.8	30.7 ± 0.4	7.4 ± 0.5
NRC602	920 ± 46	7.89 ± 0.02	24.79 ± 0.05	23.7 ± 1.3	30.4 ± 0.1	6.6 ± 1.4
NRC700	956 ± 34	7.90 ± 0.02	24.82 ± 0.06	23.5 ± 1.0	30.5 ± 0.4	7.0 ± 0.7
NRC701	971 ± 37	7.91 ± 0.01	24.85 ± 0.04	23.1 ± 0.4	30.7 ± 0.3	7.7 ± 0.1

Table 2

SPECTRUM Cigarette Physical Parameters

Product Code	Pressure Drop (Vents Open) (mm WG)	Pressure Drop (Vents Closed) (mm WG)	Tip Ventilation (%)	Total Ventilation (%)	Tobacco Length (mm)	Tobacco Mass (mg)	Rod Length (mm)
NRC100	77.1 ± 3.4	153 ± 9	58.3 ± 3.6	64.9 ± 3.4	60.7 ± 0.2	692 ± 20	83.3 ± 0.2
NRC101	72.8 ± 3.7	147 ± 7	60.7 ± 0.9	66.0 ± 0.8	60.6 ± 0.7	655 ± 29	83.2 ± 0.2
NRC102	114 ± 10	131 ± 13	15.4 ± 1.2	30.6 ± 3.3	60.5 ± 0.6	619 ± 21	83.2 ± 0.2
NRC103	128 ± 7	149 ± 9	16.5 ± 1.0	29.1 ± 2.5	60.3 ± 0.5	630 ± 25	83.1 ± 0.2
NRC104	112 ± 4	112 ± 4	0.6 ± 0.1	14.6 ± 1.6	58.9 ± 1.8	688 ± 23	84.0 ± 0.2
NRC105	120 ± 7	120 ± 7	0.5 ± 0.1	17.0 ± 1.8	60.2 ± 0.5	662 ± 22	83.2 ± 0.2
NRC200	132 ± 6	157 ± 12	18.2 ± 2.6	31.6 ± 3.5	60.0 ± 0.3	661 ± 36	83.4 ± 0.1
NRC201	135 ± 4	157 ± 5	16.6 ± 1.1	30.4 ± 2.0	60.4 ± 0.4	653 ± 59	83.2 ± 0.1
NRC300	130 ± 2	152 ± 3	16.4 ± 1.2	30.6 ± 1.9	60.1 ± 0.1	700 ± 18	82.8 ± 0.2
NRC301	123 ± 9	144 ± 12	16.9 ± 0.9	29.7 ± 2.3	60.7 ± 0.7	612 ± 13	82.9 ± 0.2
NRC302	105 ± 7	105 ± 7	0.8 ± .0.1	13.8 ± 1.5	59.0 ± 1.7	662 ± 13	83.9 ± 0.2
NRC400	122 ± 4	138 ± 3	14.2 ± 1.4	25.7 ± 1.4	60.6 ± 0.3	691 ± 20	83.2 ± 0.1
NRC401	144 ± 9	161 ± 10	13.0 ± 1.5	27.0 ± 3.0	61.4 ± 0.3	666 ± 22	83.8 ± 0.1
NRC402	134 ± 9	145 ± 9	10.2 ± 1.3	25.4 ± 2.6	61.1 ± 0.5	694 ± 34	84.1 ± 0.3
NRC404	112 ± 6	112 ± 6	0.7 ± 0.1	17.7 ± 1.5	59.7 ± 0.4	680 ± 36	83.0 ± 0.3
NRC405	108 ± 4	108 ± 4	0.5 ± 0.1	13.9 ± 2.5	60.6 ± 0.7	640 ± 48	83.6 ± 0.3
NRC500	121 ± 5	126 ± 5	4.7 ± 5.2	15.6 ± 2.1	61.2 ± 0.3	680 ± 32	83.8 ± 0.4
NRC501	118 ± 4	137 ± 5	15.8 ± 0.5	25.8 ± 1.2	60.7 ± 0.3	693 ± 24	83.2 ± 0.1
NRC600	116 ± 5	131 ± 5	13.8 ± 0.7	27.3 ± 2.9	58.9 ± 2.1	693 ± 37	84.0 ± 0.3
NRC601	127 ± 4	141 ± 4	11.9 ± 1.7	23.0 ± 2.8	60.8 ± 0.7	657 ± 15	84.1 ± 0.1
NRC602	103 ± 5	103 ± 6	0.6 ± 0.1	14.4 ± 1.9	60.1 ± 1.2	685 ± 44	83.8 ± 0.2
NRC700	113 ± 4	119 ± 4	7.2 ± 1.2	17.6 ± 1.9	60.8 ± 0.9	698 ± 28	84.3 ± 0.1
NRC701	121 ± 8	138 ± 8	15.7 ± 0.8	24.0 ± 1.0	60.8 ± 0.5	693 ± 38	83.9 ± 0.2

Mean and standard deviation of quintuplicate measurements

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Table 3

Menthol and TSNA Concentrations in the Tobacco of SPECTRUM Cigarettes (Columns 2 and 4)

Product Code	Menthol (μg/g)	Menthol (μg/cig)	NNN (ng/g)	NNK (ng/g)
NRC100	14.5 ± 1.4	14.0	1026 ± 37	52.0 ± 3.3
NRC101*	7480 ± 511	6760	1030 ± 19	49.7 ± 3.7
NRC102	11.3 ± 2.0	1.1	996 ± 21	47.8 ± 4.6
NRC103*	6720 ± 763	5980	963 ± 23	45.2 ± 3.0
NRC104	5.6 ± 0.7	5.3	972 ± 37	54.3 ± 4.2
NRC105*	7400 ± 754	6490	1013 ± 14	49.7 ± 3.2
NRC200	14.9 ± 0.5	14.7	995 ± 7	68.2 ± 3.5
NRC201*	6680 ± 545	6350	1002 ± 32	75.6 ± 6.9
NRC300	13.5 ± 0.3	13.3	1091 ± 45	93.9 ± 6.8
NRC301*	5550 ± 412	4970	1108 ± 122	88.0 ± 4.6
NRC302*	8860 ± 812	7690	1030 ± 20	75.9 ± 4.1
NRC400	9.3 ± 0.8	9.0	1217 ± 11	156 ± 7
NRC401*	6380 ± 796	6150	1244 ± 40	152 ± 6
NRC402	8.6 ± 0.6	8.2	1272 ± 104	200 ± 47
NRC404	122 ± 17	114	1254 ± 12	197 ± 11
NRC405*	8610 ± 751	7720	1148 ± 75	156 ± 19
NRC500	8.7 ± 0.9	8.1	1322 ± 50	184 ± 5
NRC501*	5540 ± 646	5470	1475 ± 28	243 ± 31
NRC600	11.1 ± 0.8	10.9	1732 ± 75	262 ± 22
NRC601*	7540 ± 764	7130	1591 ± 89	249 ± 22
NRC602*	7960 ± 789	7540	1543 ± 43	232 ± 14
NRC700	8.4 ± 0.9	8.7	1616 ± 90	517 ± 140
NRC701*	7170 ± 773	7350	1506 ± 39	302 ± 18

(Mean ± standard deviation of quintuplicate analyses of each product). Columns 4 and 5: Nitrosamine concentrations in the tobacco of SPECTRUM cigarettes (Mean ± SD of quintuplicate analyses of each product).

* Mentholated products.

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Table 4

Detectable PAH Concentrations (ng/g) From Quintuplicate Analyses of Spectrum Cigarettes.

	ACE	PHE	FLR	PYR	CHR	BBF	BKF	BEP	BAP
NRC100	<LOD	51.3±6.7	10.6±0.71	14.5±0.6	323±6	2.96±1.68	0.92±0.41	0.57±0.52	<LOD
NRC101	<LOD	49.1±11.1	10.2±0.91	14.0±1.4	265±45	3.39±1.53	1.24±0.37	<LOD	<LOD
NRC102	3.34±0.64	52.3±5.6	11.8±0.65	13.5±0.8	349±16	3.10±1.83	0.94±0.52	<LOD	<LOD
NRC103	3.60±0.27	53.4±4.5	9.94±0.44	14.6±0.4	318±30	1.49±0.83	0.99±0.36	<LOD	<LOD
NRC104	<LOD	51.7±8.6	10.8±0.93	16.2±1.0	315±10	2.02±0.82	<LOD	<LOD	<LOD
NRC105	3.74±0.13	49.7±6.4	9.93±0.78	14.8±0.8	317±8	2.07±0.93	<LOD	<LOD	<LOD
NRC200	3.20±0.26	52.5±9.3	9.55±0.72	14.4±0.7	319±12	2.35±0.78	<LOD	<LOD	<LOD
NRC201	3.45±0.14	56.4±8.5	9.91±1.17	15.0±0.9	306±32	2.29±0.54	0.71±0.14	<LOD	<LOD
NRC300	<LOD	58.5±8.8	9.61±1.20	14.1±1.2	285±34	1.17±0.31	<LOD	<LOD	<LOD
NRC301	<LOD	59.7±10.6	8.43±1.16	13.3±0.4	303±51	1.80±1.58	<LOD	<LOD	<LOD
NRC302	<LOD	58.1±9.3	10.3±0.63	14.6±1.4	283±63	1.62±1.02	<LOD	<LOD	<LOD
NRC401	4.23±0.27	47.1±4.1	10.2±0.79	15.3±1.1	234±21	<LOD	<LOD	0.64±0.27	<LOD
NRC402	3.99±0.29	47.5±5.9	11.6±0.41	16.3±0.7	260±9	<LOD	<LOD	<LOD	<LOD
NRC404	3.50±1.63	45.3±9.5	9.82±0.44	16.6±1.4	193±8	1.77±0.31	<LOD	<LOD	<LOD
NRC405	<LOD	42.4±3.0	10.9±0.35	15.7±0.9	239±27	1.20±0.54	<LOD	<LOD	<LOD
NRC500	3.59±0.24	51.6±6.2	11.4±1.03	14.8±0.3	261±7	<LOD	<LOD	<LOD	<LOD
NRC501	3.80±0.09	50.4±3.6	10.0±0.65	12.7±1.4	259±5	<LOD	<LOD	<LOD	<LOD
NRC600	<LOD	32.7±6.9	8.06±0.61	11.5±0.6	220±27	<LOD	<LOD	<LOD	<LOD
NRC601	<LOD	37.7±6.5	8.89±0.49	11.3±0.5	240±9	<LOD	<LOD	<LOD	<LOD
NRC602	3.59±0.29	40.1±1.1	10.3±0.36	12.3±1.3	254±5	<LOD	<LOD	<LOD	<LOD
NRC700	<LOD	46.5±12.5	8.39±0.95	8.47±0.7	339±14	5.10±3.09	1.46±1.03	<LOD	<LOD
NRC701	<LOD	41.4±5.5	7.78±0.76	8.75±1.1	316±53	4.12±1.86	1.53±0.51	<LOD	<LOD
LOD >	3.15	9.94	5.65	0.03	2.03	1.06	0.63	0.42	1.02
Low Std Calibrator >	2.57	8.97	4.57	3.37	1.86	0.96	0.41	0.33	0.83

Key: Acenaphthene (ACE), benzo[a]pyrene (BAP), benzo[e]pyrene (BEP), chrysene (CHR), fluoranthene (FLR), phenanthrene (PHE), and pyrene (PYR), benzo[b]fluoranthene (BBF), benzo[k]fluoranthene (BKF)

Table 5

Nicotine and minor alkaloid concentrations

Product Code	NIC (mg/g)	NNIC (μg/g)	MYOS (μg/g)	ANAB (μg/g)	ANAT (μg/g)	ISONIC (μg/g)
NRC100	0.31 ± 0.01	508 ± 29	16.5 ± 1.1	6.7 ± 0.3	32.6 ± 2.9	3.6 ± 0.2
NRC101	0.31 ± 0.00	534 ± 40	18.1 ± 1.4	6.7 ± 0.5	33.1 ± 2.7	3.5 ± 0.2
NRC102	0.31 ± 0.01	512 ± 21	16.2 ± 1.1	6.5 ± 0.4	31.7 ± 2.7	3.4 ± 0.2
NRC103	0.28 ± 0.01	472 ± 24	14.8 ± 1.0	5.9 ± 0.4	29.7 ± 2.7	3.2 ± 0.1
NRC104	0.33 ± 0.01	478 ± 20	15.9 ± 1.4	6.0 ± 0.3	30.3 ± 1.9	3.7 ± 0.2
NRC105	0.31 ± 0.01	553 ± 50	18.0 ± 1.1	6.9 ± 0.4	33.9 ± 2.9	3.4 ± 0.1
NRC200	0.93 ± 0.03	498 ± 32	15.5 ± 1.0	12.6 ± 2.7	78.0 ± 5.3	5.3 ± 0.2
NRC201	1.00 ± 0.03	534 ± 18	15.4 ± 1.4	13.0 ± 1.8	84.6 ± 14	5.5 ± 0.4
NRC300	1.90 ± 0.05	520 ± 28	14.8 ± 1.4	20.1 ± 2.5	139 ± 16	8.0 ± 0.4
NRC301	1.97 ± 0.03	544 ± 23	16.0 ± 2.1	21.1 ± 3.6	142 ± 26	8.0 ± 0.2
NRC302	2.05 ± 0.03	593 ± 58	18.3 ± 1.9	21.5 ± 2.5	162 ± 19	8.5 ± 0.5
NRC400	4.57 ± 0.07	632 ± 48	18.6 ± 2.0	47.0 ± 4.8	330 ± 37	14.2 ± 1.1
NRC401	4.81 ± 0.16	670 ± 73	18.4 ± 2.0	54.8 ± 10.3	378 ± 71	14.5 ± 1.3
NRC402	5.36 ± 0.12	681 ± 60	20.0 ± 2.0	53.9 ± 12.4	415 ± 83	18.3 ± 1.6
NRC404	7.06 ± 0.15	711 ± 52	18.2 ± 2.2	67.1 ± 10.4	489 ± 79	19.9 ± 2.1
NRC405	5.19 ± 0.09	729 ± 98	20.5 ± 1.3	55.6 ± 6.8	432 ± 41	18.1 ± 1.3
NRC500	11.1 ± 0.1	765 ± 60	19.8 ± 1.4	101 ± 10	814 ± 89	30.5 ± 2.1
NRC501	10.0 ± 0.2	786 ± 40	17.8 ± 3.3	113 ± 16	825 ± 108	29.9 ± 4.7
NRC600	15.7 ± 0.3	1020 ± 94	19.5 ± 2.5	163 ± 19	1344 ± 151	41.8 ± 4.0
NRC601	17.3 ± 0.6	1235 ± 133	28.7 ± 5.7	205 ± 47	1683 ± 368	54.6 ± 9.9
NRC602	14.2 ± 0.5	1070 ± 136	24.8 ± 3.5	178 ± 24	1464 ± 190	44.5 ± 4.9
NRC700	23.4 ± 0.6	1218 ± 157	22.2 ± 2.7	242 ± 39	2124 ± 331	64.9 ± 6.7
NRC701	25.0 ± 1.2	1221 ± 110	20.6 ± 1.1	231 ± 49	2027 ± 418	61.1 ± 6.6

(Mean ± standard deviation of quintuplicate analyses of each product).

Key: NIC = nicotine, NNIC = nornicotine, MYOS = myosmine, ANAB = anabasine, ANAT = anatabine, ISONIC = isonicotine.

Table 6

Ammonia and Toxic Metal and Metalloid Concentrations in SPECTRUM Cigarette Filler Tobacco

Product Code	NH_3 mg/g	Be (μg/g)	Cr (μg/g)	Mn (μg/g)	Co (μg/g)	Ni (μg/g)	As (μg/g)	Se (μg/g)	Cd (μg/g)	Hg (μg/g)	Pb (μg/g)	U (μg/g)
NRC100	3.3 ± 0.2	0.016 ± 0.002	1.08 ± 0.08	153 ± 2	0.51 ± 0.07	1.76 ± 0.19	0.105 ± 0.008	0.077 ± 0.008	0.97 ± 0.05	0.0234 ± 0.0007	0.82 ± 0.07	0.028 ± 0.002
NRC101	3.4 ± 0.1	0.015 ± 0.002	0.89 ± 0.03	158 ± 3	0.49 ± 0.04	1.53 ± 0.08	0.105 ± 0.010	0.080 ± 0.012	0.96 ± 0.04	0.0237 ± 0.0007	0.64 ± 0.04	0.026 ± 0.004
NRC102	3.3 ± 0.1	0.018 ± 0.001	1.00 ± 0.15	154 ± 5	0.49 ± 0.03	1.61 ± 0.15	0.114 ± 0.012	0.072 ± 0.006	0.91 ± 0.04	0.0235 ± 0.0007	0.63 ± 0.03	0.027 ± 0.003
NRC103	3.7 ± 0.1	0.018 ± 0.002	0.94 ± 0.18	210 ± 9	0.57 ± 0.04	1.57 ± 0.13	0.104 ± 0.012	0.090 ± 0.007	1.00 ± 0.06	0.0209 ± 0.0004	0.57 ± 0.07	0.024 ± 0.001
NRC104	3.5 ± 0.2	0.017 ± 0.002	1.05 ± 0.16	197 ± 11	0.52 ± 0.04	1.67 ± 0.18	0.118 ± 0.009	0.080 ± 0.015	0.96 ± 0.04	0.0210 ± 0.0005	0.58 ± 0.03	0.025 ± 0.002
NRC105	3.3 ± 0.1	0.018 ± 0.002	0.98 ± 0.09	208 ± 5	0.58 ± 0.03	1.73 ± 0.13	0.109 ± 0.003	0.078 ± 0.008	0.98 ± 0.03	0.0217 ± 0.0004	0.59 ± 0.06	0.025 ± 0.001
NRC200	3.5 ± 0.1	0.017 ± 0.003	1.21 ± 0.13	222 ± 3	0.58 ± 0.05	1.73 ± 0.17	0.113 ± 0.010	0.087 ± 0.006	1.00 ± 0.02	0.0211 ± 0.0007	0.55 ± 0.05	0.025 ± 0.002
NRC201	3.4 ± 0.1	0.018 ± 0.002	1.21 ± 0.09	211 ± 5	0.58 ± 0.05	1.78 ± 0.23	0.116 ± 0.010	0.088 ± 0.013	1.03 ± 0.04	0.0218 ± 0.0007	0.61 ± 0.07	0.027 ± 0.001
NRC300	3.3 ± 0.1	0.017 ± 0.001	1.13 ± 0.12	196 ± 4	0.54 ± 0.02	1.65 ± 0.08	0.106 ± 0.003	0.083 ± 0.007	0.97 ± 0.04	0.0207 ± 0.0004	0.59 ± 0.05	0.027 ± 0.002
NRC301	3.4 ± 0.1	0.018 ± 0.002	1.17 ± 0.13	214 ± 7	0.59 ± 0.04	1.72 ± 0.14	0.107 ± 0.006	0.088 ± 0.011	0.99 ± 0.04	0.0209 ± 0.0005	0.57 ± 0.03	0.026 ± 0.001
NRC302	3.6 ± 0.2	0.018 ± 0.001	1.21 ± 0.11	206 ± 7	0.57 ± 0.04	1.79 ± 0.11	0.106 ± 0.007	0.069 ± 0.005	1.02 ± 0.01	0.0229 ± 0.0009	0.61 ± 0.01	0.027 ± 0.001
NRC400	3.1 ± 0.1	0.017 ± 0.001	1.29 ± 0.20	200 ± 4	0.53 ± 0.02	1.70 ± 0.15	0.116 ± 0.007	0.082 ± 0.014	1.02 ± 0.04	0.0214 ± 0.0007	0.53 ± 0.02	0.026 ± 0.001
NRC401	3.2 ± 0.2	0.019 ± 0.002	1.05 ± 0.04	190 ± 5	0.54 ± 0.05	1.53 ± 0.08	0.106 ± 0.008	0.074 ± 0.003	0.94 ± 0.03	0.0208 ± 0.0003	0.54 ± 0.03	0.027 ± 0.001
NRC402	3.2 ± 0.1	0.019 ± 0.003	1.33 ± 0.18	188 ± 6	0.51 ± 0.02	1.71 ± 0.11	0.116 ± 0.009	0.078 ± 0.009	0.94 ± 0.06	0.0203 ± 0.0002	0.57 ± 0.06	0.034 ± 0.004
NRC404	3.1 ± 0.2	0.019 ± 0.002	1.12 ± 0.09	196 ± 3	0.50 ± 0.01	1.60 ± 0.08	0.117 ± 0.012	0.079 ± 0.007	1.00 ± 0.06	0.0213 ± 0.0007	0.53 ± 0.02	0.031 ± 0.002
NRC405	3.2 ± 0.2	0.020 ± 0.002	1.11 ± 0.11	200 ± 5	0.51 ± 0.05	1.63 ± 0.07	0.118 ± 0.005	0.079 ± 0.006	0.99 ± 0.02	0.0208 ± 0.0006	0.59 ± 0.03	0.031 ± 0.001
NRC500	2.9 ± 0.1	0.018 ± 0.003	1.12 ± 0.03	196 ± 2	0.44 ± 0.03	1.35 ± 0.11	0.126 ± 0.008	0.073 ± 0.013	0.99 ± 0.05	0.0209 ± 0.0003	0.59 ± 0.03	0.032 ± 0.002
NRC501	2.8 ± 0.1	0.017 ± 0.002	1.34 ± 0.07	176 ± 3	0.47 ± 0.02	1.65 ± 0.07	0.121 ± 0.005	0.072 ± 0.007	0.97 ± 0.03	0.0206 ± 0.0004	0.54 ± 0.02	0.032 ± 0.003
NRC600	2.4 ± 0.1	0.014 ± 0.002	1.25 ± 0.08	180 ± 2	0.43 ± 0.03	1.58 ± 0.08	0.145 ± 0.005	0.067 ± 0.008	0.98 ± 0.06	0.0213 ± 0.0017	0.50 ± 0.01	0.031 ± 0.002
NRC601	2.7 ± 0.1	0.014 ± 0.001	1.14 ± 0.12	169 ± 6	0.41 ± 0.04	1.42 ± 0.11	0.143 ± 0.008	0.069 ± 0.010	0.95 ± 0.04	0.0203 ± 0.0014	0.45 ± 0.04	0.030 ± 0.002
NRC602	2.9 ± 0.1	0.016 ± 0.003	1.18 ± 0.12	209 ± 5	0.48 ± 0.04	1.54 ± 0.09	0.134 ± 0.008	0.064 ± 0.010	1.04 ± 0.07	0.0192 ± 0.0005	0.50 ± 0.01	0.031 ± 0.002
NRC700	2.2 ± 0.1	0.013 ± 0.001	1.17 ± 0.11	163 ± 5	0.36 ± 0.02	1.50 ± 0.10	0.168 ± 0.014	0.043 ± 0.008	1.08 ± 0.05	0.0181 ± 0.0005	0.43 ± 0.02	0.034 ± 0.003
NRC701	2.2 ± 0.1	0.013 ± 0.002	1.29 ± 0.10	171 ± 1	0.38 ± 0.01	1.64 ± 0.06	0.177 ± 0.009	0.048 ± 0.006	1.11 ± 0.05	0.0183 ± 0.0009	0.40 ± 0.01	0.036 ± 0.001

Table 6. Mean results ± standard deviation for quintuplicate analyses of SPECTRUM cigarette filler tobacco for 11 inorganic analytes (μg/g